

Short Communication

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Preliminary characterization of the oligosaccharide component of arylsulfatase B from human placenta*

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Isoelectric focusing of homogenous arylsulfatase B from human placenta pointed to the presence of enzymatically active and inactive forms of high pI (pH 9–8) and of lower pI (pH 6.5–5.5). Glycan chain analysis performed with the use of a Glycan Differentiation Kit showed that basic forms of arylsulfatase B from human placenta contained mostly high mannose/hybrid type glycans, with 6-O-L-fucose bound to the innermost N-acetylglucosamine residue, whereas acidic forms of the enzyme contained complex type glycans containing fucose and sialic acid. However, the latter forms constitute a small percentage of the total carbohydrate component. Lectin affinity chromatography of the native enzyme confirmed the presence of a core fucose and a sialic acid.

Arylsulfatase B (EC 3.1.6.1) from human tissues, a soluble lysosomal sulfohydrolase, is a glycoprotein [1]. Based on cDNA of human arylsulfatase B it has been shown that the enzyme has six potential N-glycosylation sites [2, 3] of which presumably no more than four are glycosylated [3, 4]. The enzyme from human tissues is highly heterogenous with respect to charge [5–9]. The results of isoelectric focusing of human arylsulfatase B indicated that the majority of enzymatically active forms

were of high pI (pH 8–9) [7, 10, 11]. However, the minor active, probably phosphorylated forms of lower pI (pH 6–7) were also observed [7, 12, 13]. As a lysosomal soluble enzyme, arylsulfatase B should possess high mannose type glycans [14]. Neurath *et al.* [4] suggested the presence of complex or hybrid type glycans as well. The carbohydrate component of the enzyme present in tumor tissues has been shown undergo changes [8, 12, 13, 15]. Despite numerous studies on arylsulfatase B, the de-

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¹Abbreviations: AAA, *Aleuria aurantia* agglutinin; DIG, digoxigenin; DSA, *Datura stramonium* agglutinin; Fuc, L-fucose; Gal, galactose; GlcNAc, N-acetylglucosamine; GNA, *Galanthus nivalis* agglutinin; IEF, isoelectric focusing; MAA, *Maackia amurensis* agglutinin; Man, mannose; NeuAc, neuraminic acid; LCA, *Lens culinaris* agglutinin; PAGE, polyacrylamide gel electrophoresis; RCA, *Ricinus communis* agglutinin; SNA, *Sambucus nigra* agglutinin.

tailed structure of its oligosaccharides has not been determined, therefore, studies to elucidate some of their major features were performed.

MATERIALS AND METHODS

Materials. A Glycan Differentiation Kit containing the digoxigenin (DIG)¹-labelled lectins: *Galanthus nivalis* agglutinin (GNA), the lectin specific for terminal Man (α 1-2, α 1-3 or α 1-6)-Man units; *Sambucus nigra* agglutinin (SNA), the lectin specific for NeuAc(α 2-6)Gal; *Maackia amurensis* agglutinin (MAA), the lectin specific for NeuAc(α 2-3)Gal; *Datura stramonium* agglutinin (DSA), the lectin specific for Gal(β 1-4)GlcNAc; and *Aleuria aurantia* agglutinin (AAA), the lectin specifically binding Fuc(α 1-6)GlcNAc, was purchased from Boehringer (Mannheim). *Ricinus communis* agglutinin-agarose (RCA), the lectin specific for terminal β -D-Gal and *Limulus polyphemus* lectin specifically binding to NeuAc were obtained from Sigma Chemical Co. Neuraminidase from *Vibrio cholerae* was purchased from Calbiochem-Boehringer Group. *Lens culinaris* agglutinin (LCA), the lectin specific for Man residue in the presence of Fuc(α 1-6)GlcNAc as well as chromatography resins and media were purchased from Pharmacia LKB. Nitrocellulose was from Schleicher and Schuell. Servalyt Precotes 3-10 and Servalyt PreNets 3-10 were from Serva (Germany). All other reagents were of analytical grade.

Purification of arylsulfatase B. Arylsulfatase B was purified from human placenta. Frozen tissue was thawed and homogenized in an Ultra-Turrax in three volumes of ice-cold 20 mM Tris/HCl, pH 7.5, containing 0.1% Triton X-100. Ammonium sulphate, 25% saturation followed by 75% saturation was used to precipitate the protein from supernatant. The precipitate was dissolved in 20 mM sodium acetate, pH 5.8, dialysed against the same buffer and applied to CM Sephadex C-50. Arylsulfatase B was eluted with a linear NaCl gradient (0-1 M), dialysed against 20 mM Tris/HCl, pH 7.5 and bound to Con A-Sepharose. The enzyme, eluted with 0.3 M mannose in starting buffer containing 1 M NaCl, was further separated on Sepharose S-300 in 50 mM Tris/HCl, pH 7.5 with 0.1 M NaCl followed by passing

without binding through DEAE Sepharose in 50 mM Tris/HCl, pH 7.5.

Enzymatic activity and protein concentration were determined according to routinely used procedures [16, 17]. Specific activity was expressed in μ moles of *p*-nitrocatechol per min/mg of protein.

On polyacrylamide gel electrophoresis at pH 4.5 [18] the protein migrated as a single band (Coomassie R-250 staining) corresponding to enzymatic activity (the reaction with *p*-nitrocatechol sulfate [16]). The immunochemical identity of the band was confirmed after electrophoretic transfer onto nitrocellulose [19] using rabbit antiserum against human placenta arylsulfatase B kindly supplied by Dr. K. von Figura, Göttingen (Germany).

The final preparation of arylsulfatase B was also chromatofocused on PBE 94 gel in the range of pH 9-6 with polybuffer 96 according to manufacturer's instructions (Pharmacia Fine Chemicals 1980).

Isoelectric focusing. This was carried out between pH 3 and 10 on an Desaga Desaphor HF apparatus using Servalyt Precotes. Servalyt PreNets (pH 3-10) were used when the focusing was followed by electrophoretic transfer onto nitrocellulose [20]. The protein on the gel and nitrocellulose was stained with Coomassie R-250 and Amido Black, respectively. The enzyme activity on the nitrocellulose was visualized with *p*-nitrocatechol sulfate [16].

Neuraminidase treatment. Arylsulfatase B was desialylated with neuraminidase from *V. cholerae* in 0.5 M acetate buffer, pH 5.0. Samples of the enzyme (20 μ g) were incubated with 50 mU of sialidase at 37°C for 20 h.

Glycan chain analysis. Glycan chain analysis of native placental arylsulfatase B was performed alternatively:

- 1, with the use of a glycan Differentiation Kit [21] on the enzyme electrophoretically transferred onto nitrocellulose after isoelectric focusing on Servalyt PreNets (pH 3-10). In this test all the lectins used became bound to their respective standards, as checked by a dot-blot test;
- 2, by means of lectin affinity chromatography on LCA-agarose, LIM-Sepharose and RCA I-agarose. Chromatography was carried out as described elsewhere [22]. From LIM-Sepharose the enzyme was eluted with 0.2 M sialic

acid in starting buffer (10 mM Tris/HCl, pH 8.0, containing 0.15 M NaCl).

RESULTS AND DISCUSSION

Arylsulfatase B was purified from human placenta. The enzyme with specific activity of 40 U/mg protein was electrophoretically (PAGE at pH 4.5) and immunochemically (western blot) homogenous, but showed heterogeneity upon isoelectric focusing. The majority of the enzyme protein and activity focused in the range of pH 8–9. However, the forms focusing in this pH range differed in enzymatic activity. The most active form was of the highest pI while the others showed little or no activity (Fig. 1 and 2). Enzymatically active forms that represented trace amounts of arylsulfatase B were also observed between pH 6.5 and 5.5 (Fig. 1). These results are not surprising since high heterogeneity of arylsulfatase B with respect to charge has also been reported for the enzyme from various human tissues [5–13].

The carbohydrate component of the enzyme was studied with the use of digoxigenin-labelled lectins. The basic forms showed a positive reaction only with GNA and AAA, lectins specific for mannose and core fucose, respectively. This suggested the presence of high mannose/hybrid type glycans with 6-O-L-fucose bound to the innermost *N*-acetylglucosamine

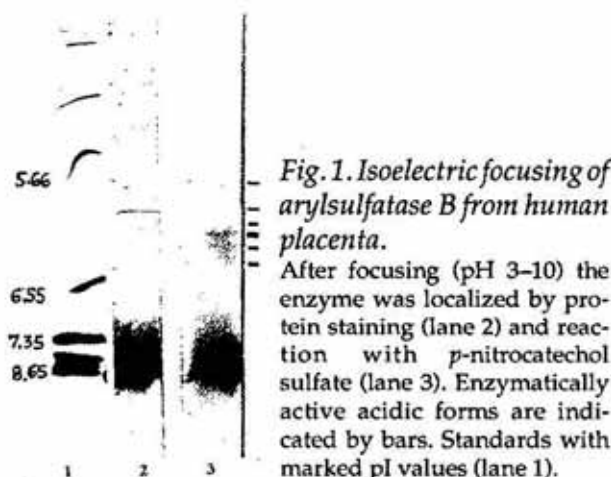


Fig. 1. Isoelectric focusing of arylsulfatase B from human placenta.

After focusing (pH 3–10) the enzyme was localized by protein staining (lane 2) and reaction with *p*-nitrocatechol sulfate (lane 3). Enzymatically active acidic forms are indicated by bars with marked pI values (lane 1).

residue (Fig. 2). The basic forms of arylsulfatase B were further separated from the rest of protein using chromatofocusing in the pH range of 9–6 (Fig. 3A) and their glycans analyzed after isoelectric focusing. The results confirmed that the only glycans present in basic forms of the enzyme, regardless of their activity, were of the high mannose/hybrid type with a core fucose (Fig. 3B). The six dominating basic forms differed in the intensity of the reactions with GNA and AAA. The most active form (pI about 9) contained relatively less fucose than the other forms (Fig. 3B).

Unlike the basic forms, minor acidic forms that focused between pH 6.5 and 5.5 reacted with digoxigenin-labelled SNA and DSA, lectins specific for sialic acid and terminal galac-

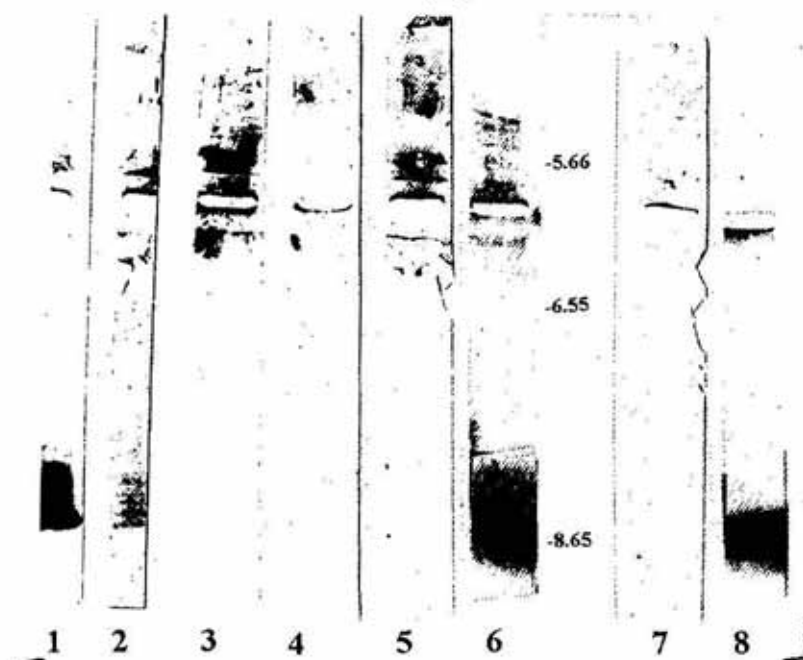


Fig. 2. Probing of arylsulfatase B with digoxigenin-labelled lectins.

The enzyme before (lanes 1–6) and after desialylation (lanes 7, 8) was focused (pH 3–10), transferred onto nitrocellulose and probed with lectins: GNA (1); AAA (2); SNA (3); MAA (4); DSA (5); MAA/SNA (7). Protein staining (6, 8).

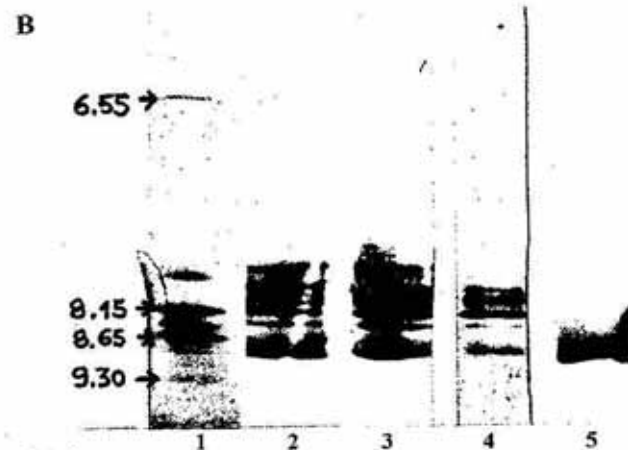
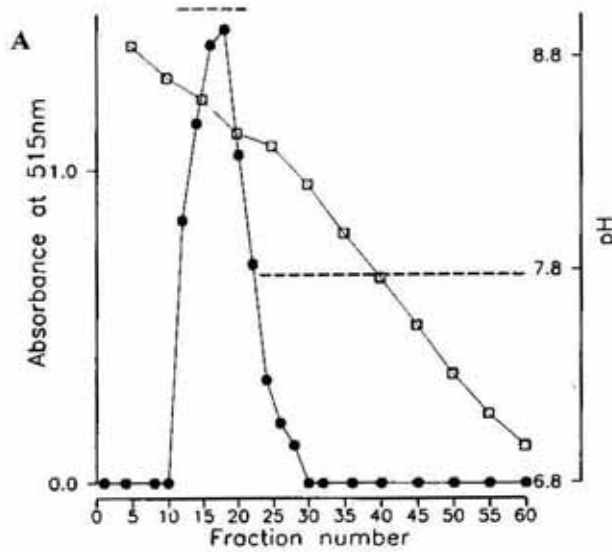


Fig. 3. Chromatofocusing of arylsulfatase B from human placenta (A) followed by isoelectric focusing and probing with digoxigenin-labelled lectins (B).

The enzyme was chromatofocused as described under Materials and Methods. (A) Absorbance at 515 nm (enzymatic activity), solid dots; pH profile, open squares. (B) The active fractions were pooled, focused (pH 3–10), transferred onto nitrocellulose and stained for protein (lane 2), probed with GNA (lane 3), AAA (lane 4) and localized by the reaction with *p*-nitrocatechol sulfate (lane 5). pH Values indicated on the left were evaluated using marker proteins (lane 1).

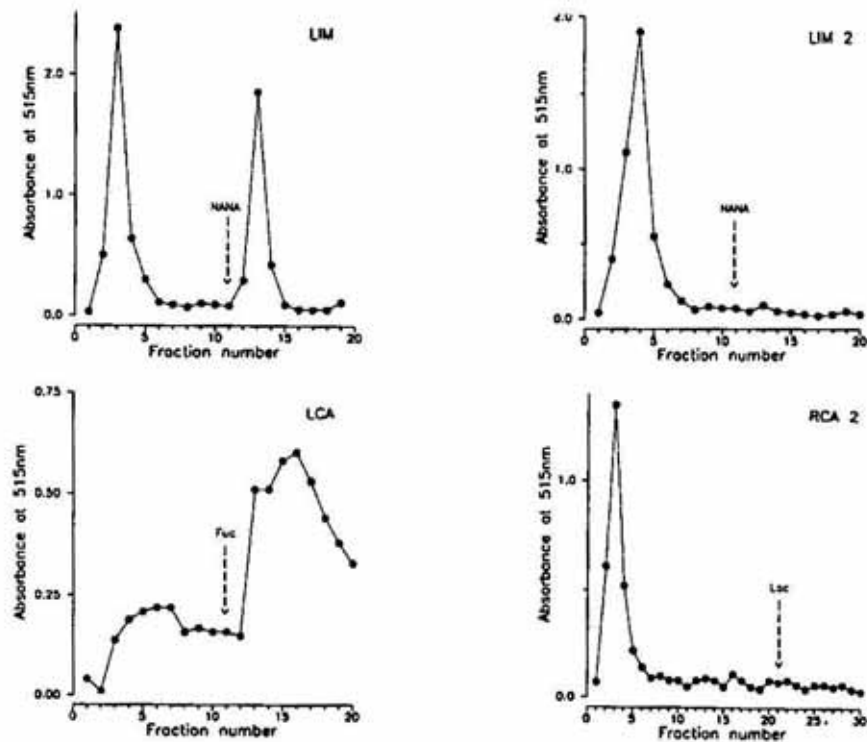


Fig. 4. Lectin affinity chromatography of human placental arylsulfatase B.

Elution patterns of enzymatically active arylsulfatase B chromatographed on LIM-Sepharose (LIM) and LCA-agarose (LCA). The desialylated enzyme was chromatographed on LIM-Sepharose (LIM2) and RCA I-agarose (RCA2). The addition of eluting monosaccharide was marked by arrows.

tose, respectively. They also showed a positive reaction with AAA but did not react with GNA (Fig. 2). This indicates that acidic forms of arylsulfatase B contain complex type glycans. These forms and therefore their glycans represent only a small percentage of the enzyme from human placenta. After treatment of the enzyme with neuraminidase the acidic forms of arylsulfatase B were no longer observed (Fig. 2).

Native arylsulfatase B was passed through LCA-agarose, LIM-Sepharose and RCA I-agarose to further characterize its oligosaccharide component. The enzyme partially bound to LCA-agarose and LIM-Sepharose and was eluted with the competing ligand. Desialylated arylsulfatase B did not bind to LIM-Sepharose (Fig. 4). These results confirmed the presence of fucose and sialic acid. The activity of the fraction bound to LIM-Sepharose was higher than expected in relation to the amount of protein bearing the complex type glycans (Figs. 1 and 4). Those forms, although present in trace amounts, might have high specific activity. The meaning of this observation is not clear at present. The desialylated enzyme passed through RCA I-agarose without retardation. However, not the whole activity applied to the column was recovered (Fig. 4). No activity was eluted, either, with the competing ligand. This was somewhat surprising since complex type oligosaccharides after desialylation should have bound to this lectin [23]. Further studies are required to explain this observation.

The study showed that the majority of glycans of arylsulfatase B from human placenta are of the high mannose/hybrid type with a core fucose. Similar results were recently reported for arylsulfatase A from human platelet [24] and placenta [22]. Sialylated glycans that could participate in creation of charge heterogeneity of arylsulfatase B were also observed. However, they represented a small percentage of the total carbohydrate component as it was the case with human arylsulfatase A [22].

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